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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. **EV 335774769 US**

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Michael A.	**Brownlee**	**New York, NY**

Additional inventors are being named on the -- *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

GLP-1 (9-36) METHODS AND COMPOSITIONS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* **32**
☑ Drawing(s) *Number of Sheets* **4**
☐ Application Date Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify) specification cover page; cover letter; return receipt postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees.
☐ The Director is herby authorized to charge **additional** fees or credit any overpayment to Deposit Account Number: **01-1785**
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Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME **Elie H. Gendloff**

TELEPHONE **212-336-8108**

Date **December 12, 2003**

REGISTRATION NO. **44,704** *(if appropriate)*

Docket Number: **96700/843**

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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December 12, 2003

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
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Mail Stop Provisional Patent Application
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Re: Rule 1.53(c) Provisional Patent Application Claiming Small Entity Status
Title: GLP-1 (9-36) METHODS AND COMPOSITIONS
Inventor: Michael A. Brownlee
Our File: 96700/843

Dear Sir:

Pursuant to 37 C.F.R. §1.53(c), enclosed please find the following documents for filing with the above-identified provisional patent application claiming small entity status in the name of Michael Brownlee, entitled GLP-1 (9-36) METHODS AND COMPOSITIONS, comprising the following:

1. Provisional Application For Patent Cover Sheet (Form PTO/SB/16) (1 page);

2. Provisional patent application, including: application cover page (1 page), specification (22 pages), claims (9 pages), abstract (1 page), and drawings (4 sheets);

3. Amster, Rothstein & Ebenstein LLP check in the amount of $80.00 to cover the provisional application filing fee for small entity status; and

4. Return receipt postcard.

Please acknowledge receipt of the enclosed papers by stamping the enclosed postcard and returning the same to us.

Respectfully submitted,

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Dated: December 12, 2003
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Elie H. Gendloff
Registration No. 44,704

AR&E Docket No. 96700/843

New U.S. Provisional Patent Application (Small Entity Status)

Title: GLP-1 (9-36) METHODS AND COMPOSITIONS

Inventor: Michael A. Brownlee

Express Mail EV 335774769 US

GLP-1 (9-36) Methods and Compositions

Background

(1) Field of the Invention

The present invention generally relates to treatments for complications of diabetes and other disorders involving hyperglycemia. More specifically, the invention relates to treatments that reduce reactive oxygen formation induced by hyperglycemia or free fatty acids.

(2) Description of the Related Art

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PCT Patent Application Publication WO 02/085406.

U.S. Patent Application Publication 2003/0073626 A1.

U.S. Provisional Patent Application No. 60/474,520.

Diabetes causes a variety of pathological changes in capillaries, arteries, and peripheral nerves. Diabetes-specific microvascular disease is the leading cause of blindness, renal failure, and nerve damage, and diabetes-associated atherosclerosis causes high rates of heart attack, stroke, and limb amputation. Seventy percent of all heart attack patients have either diabetes or impaired glucose tolerance.

Large prospective clinical studies in both type 1 and type 2 diabetic patients have shown that there is a strong relationship between the level of

hyperglycemia and both onset and progression of diabetic microvascular complications in the retina, kidney, and peripheral nerve (DCCTRG, 1993; UKPDSG, 1998). Hyperglycemia also appears to have an important role in the pathogenesis of diabetic macrovascular disease (UKPDSG, 1998; Wei et al., 1998). Four major molecular mechanisms have been implicated in hyperglycemia-induced tissue damage: activation of protein kinase C (PKC) isoforms via de novo synthesis of the lipid second messenger diacylglycerol (DAG), increased hexosamine pathway flux, increased advanced glycation endproduct (AGE) formation, and increased polyol pathway flux. In aortic endothelial cells, hyperglycemia also activates the proinflammatory transcription factor NFκB. Recently, it has been shown that all of these mechanisms reflect a single hyperglycemia-induced process: overproduction of superoxide (or reactive oxygen) by the mitochondrial electron transport chain (Brownlee, 2001; Nishikawa et al., 2000).

Glucagon-like peptide-1 (GLP-1) is synthesized in intestinal endocrine cells, in response to nutrient ingestion (Orskov et al., 1994), by differential processing of pro- glucagon into 2 principal major molecular forms - GLP-1(7-36)amide and GLP-1(7-37). The peptide was first identified following the cloning of cDNAs and genes for proglucagon in the early 1980s.

Initial studies of GLP-1 biological activity in the mid 1980s utilized the full length N-terminal extended forms of GLP-1 (1-37 and 1-36^{amide}). These larger GLP-1 molecules were generally found to be devoid of biological activity. In 1987, 3 independent research groups demonstrated that removal of the first 6 amino acids resulted in a shorter version of the GLP-1 molecule with substantially enhanced biological activity.

The majority of circulating biologically active GLP-1 is found in the GLP-1(7-36)amide form. The known major biological effects of GLP-1 (7-36) include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, inhibition of glucagon secretion and gastric emptying, and inhibition of food intake (Drucker, 1998). The finding that GLP-1 lowers blood

glucose in patients with diabetes, taken together with suggestions that GLP-1 may restore β cell sensitivity to exogenous secretagogues, suggests that augmenting GLP-1 signaling is a useful strategy for treatment of diabetic patients. Mounting evidence strongly suggests that GLP-1 signaling regulates islet proliferation and islet neogenesis (Buteau et al., 1999).

GLP-1 is rapidly inactivated to its degradation product GLP-1 (9-36) by the enzyme dipeptidyl peptidase IV (DPP IV). DPP IV-mediated inactivation is a critical control mechanism for regulating the biological activity of GLP-1 in vivo in both rodents and humans (Mentlelin et al., 1993; Kieffer et al., 1995; Deacon et al., 1995a and b). Several studies have also implicated a role for neutral endopeptidase 24.11 in the endoproteolysis of GLP-1 (Hupe-Sodmann et al., 1995; Hupe-Sodmann et al., 1997).

DPP IV inhibitors, and more-slowly degrading analogs of GLP-1 (7-36) are currently being developed for therapeutic purposes. GLP-1 analogues that are resistant to DPP IV cleavage are more potent *in vivo*. An example of a naturally occurring DPP IV-resistant GLP-1 analogue is lizard exendin-4 (Edwards et al., 2001).

There have been a few reports indicating that GLP-1 (9-36) has some biological activity. Deacon et al., 2002, provides data indicating that GLP-1 (9-36) reduces total blood glucose somewhat 10-20 minutes after glucose infusion. This small reduction in blood glucose would not be expected to affect hyperglycemia-induced reactive oxygen formation, however. Additionally, Wettergren et al., 1998, found no effect from GLP-1 (9-36) on atrial motility. Neither Deacon et al. nor Wettergren et al. indicate that GLP-1 (9-36) is capable of inhibiting hyperglycemia-induced or fatty acid-induced reactive oxygen formation.

Three patent publications, WO 03/061362, WO 02/085406 and US 2003/0073626, have claims to therapeutic treatments using GLP-1 (9-36). However, those publications do not provide an enabling disclosure of any GLP-1 (9-36) activity.

There is thus a need for new treatments that reduce or eliminate hyperglycemia-induced reactive oxygen species, in order to reduce complications of diabetes. There is also a need to determine whether GLP-1(9-36) has any clinically significant activity. The present invention addresses both of these needs.

Summary of the Invention

Accordingly, the inventor has discovered that GLP-1 (9-36) inhibits hyperglycemia-induced reactive oxygen formation in mammalian cells. Based on this discovery, methods and compositions are provided that are useful for inhibiting various disorders caused by reactive oxygen.

Thus, in some embodiments, the invention is directed to methods of inhibiting hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in a mammalian nerve cell, renal mesangial cell, β cell, adipocyte, or, preferably an endothelial cell or hepatocyte. The methods comprise treating the cell with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the cell.

In other embodiments, the invention is directed to methods of inhibiting the development of disease due to diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance in a mammal, or conditions resulting therefrom. The methods comprise treating the mammal with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal.

The invention is also directed to methods of reducing hyperglycemia-induced or free fatty acid-induced inactivation of prostacyclin synthase in a mammal. The methods comprise treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal.

The invention is further directed to methods of inhibiting hyperglycemia-induced or free fatty acid-induced decrease in endothelial nitric oxide synthetase (eNOS) activity in an endothelial cell. The methods comprise treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced decrease in eNOS activity in the cell.

In additional embodiments, the invention is directed to isolated and purified GLP-1 (9-36) consisting essentially of a sequence selected from the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16. Compositions comprising these GLP-1 (9-36) forms, in a pharmaceutically acceptable excipient, are also provided.

Brief Description of the Drawings

FIG. 1 is a graph of experimental results establishing that GLP-1 (9-36) prevents hyperglycemia-induced reactive oxygen production in vascular endothelial cells.

FIG. 2 is a graph of experimental results establishing that GLP-1 (9-36) prevents hyperglycemia-induced decreases in endothelial nitric oxide synthase activity in vascular endothelial cells.

FIG. 3 is a graph of experimental results establishing that GLP-1 (9-36) prevents diabetes-induced inactivation/inhibition of prostacyclin synthase in diabetic mouse aortas.

FIG. 4 is a graph of experimental results establishing that GLP-1 (9-36) prevents hyperglycemia-induced reactive oxygen production in hepatocytes.

Detailed Description of the Invention

The present invention is based on the discovery that GLP-1 (9-36) inhibits hyperglycemia-induced reactive oxygen formation in mammalian cells. This discovery leads to the use of GLP-1 (9-36) and similar compounds for the treatment of complications caused by reactive oxygen.

Thus, in some embodiments, the invention is directed to methods of inhibiting hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in a mammalian cell. The methods comprise treating the cell with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the cell. The cell is preferably part of a living mammal.

In preferred embodiments, the reactive oxygen formation is hyperglycemia induced, however, since free fatty acids are known to induce reactive oxygen (See, e.g., U.S. Provisional Patent App. No. 60/474,520 and references cited therein), that induction would also be expected to be affected by GLP-1 (9-36).

The cell is any cell that is capable of producing reactive oxygen in response to hyperglycemia or free fatty acids. The cell is preferably a cell that is affected by reactive oxygen to cause complications associated with hyperglycemia or free fatty acids, for example a nerve cell, a renal mesangial cell, a β cell, an adipocyte, an endothelial cell or a hepatocyte.

In some preferred embodiments, the cell is an endothelial cell, preferably a vascular endothelial cell. The endothelial cell is preferably in a mammal (most preferably a human) that has or is at risk for having diabetes, impaired glucose intolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance. The methods would also be useful for a critically ill mammal, since hyperglycemic mechanisms are risk factors in critically ill patients, even when they were not diabetic (Van den Berghe et al., 2001). Complications from chronic ischemia would also be usefully treated with any of the various GLP-1 (9-36) forms.

In other preferred embodiments, the cell is a hepatocyte, preferably in a living mammal that has or is at risk for ischemia/reperfusion injury, endotoxin injury, or alcoholic liver disease. See also Example 4, showing that treatment of hepatocytes with GLP-1 (9-36) also beneficially reduces hyperglycemia-induced reactive oxygen formation.

In additional preferred embodiments, the cell is a β cell, preferably in a living mammal that has or is at risk for impaired glucose-stimulated insulin secretion.

In these methods, the GLP-1 (9-36) preferably has the sequence of SEQ ID NO:1. However, the term "GLP-1 (9-36)" is not limited to SEQ ID NO:1, but could also include any of SEQ ID NO:2-16, since each of those sequences are expected to be useful for reducing reactive oxygen formation induced by hyperglycemia or free fatty acids. Specifically, SEQ ID NO:2 is naturally occurring GLP-1 (9-37), i.e., GLP-1 (9-36) along with the 37th amino acid of GLP-1, Gly.

GLP-1 (9-36) can also usefully comprise an additional arginine (GLP-1 (9-36 + arg37)) (SEQ ID NO:3) to raise the isoelectric point, giving the peptide reduced solubility and slower degradation, similar to insulin glargine, a long-acting insulin derivative. Other amino acid changes that raise the isoelectric point towards physiological pH would also have slower degradation.

Acylation of the ϵ-amino group of Lys B29 in insulin with myristoylic acid promotes reversible binding of insulin to albumin, thereby delaying absorption from the subcutaneous injection site. With GLP-1 (9-36), similar acylation could be accomplished at Lys 26, and/or Lys 34, in combination with any of the previously described GLP-1 (9-36) (SEQ ID NO:4-16). Such peptides could be, e.g., injected subcutaneously, or administered by inhalation of modified peptides encapsulated in a biodegradable polymer as described in Edwards, D., et al., 1997; VanBever, R. et al., 1999; and Hrkach, 2000.

Additionally, each of the sequences SEQ ID NO:1-16 could also be an amide, since the amide of GLP-1 (7-36) is the naturally occurring active form of this peptide.

The GLP-1 (9-36) forms described above can be made by any known method, e.g., enzymatic digestion of a larger form, for example using DPP IV, expression of the peptide using an expression vector comprising a nucleotide sequence that encodes the GLP-1 (9-36), or, preferably, by chemical synthesis.

The GLP-1 (9-36) can also be a peptidomimetic, as are known in the art.

In some embodiments, it may also be useful to evaluate the effectiveness of these methods by known methods, for example by directly measuring reactive oxygen in the cell.

Another method of evaluating the effectiveness of these methods is by measuring prostacyclin synthase activity in the endothelial cell, since prostacyclin synthase is very sensitive to inactivation by reactive oxygen (see, e.g., Example 3). The prostacyclin synthase can be measured by any known method. A preferred method is measuring the formation of 6-keto-$PGF_{1\alpha}$ (Example 3).

This invention could be used in both prophylactic and therapeutic regimens. For prophylactic use, patients with Type I or Type II diabetes, impaired glucose tolerance, the metabolic syndrome, or stress hyperglycemia, would continuously take the pharmaceutical GLP-1 (9-36) composition along with their usual medical regimen to diminish complications due to the reactive oxygen. For therapeutic use, these inhibitors would be administered at the time of the ischemic event to decrease subsequent morbidity and mortality.

When the endothelial cell is in a living mammal, the GLP-1 (9-36) composition can be formulated without undue experimentation for administration to the mammal, including humans, as appropriate for the particular application. Additionally, proper dosages of the GLP-1 (9-36) compositions can be determined without undue experimentation using standard dose-response protocols. Preferred methods of administration include administration intravenously and by subcutaneous infusion pump. However, the invention is not narrowly limited to any particular methods of administration.

Accordingly, the compositions designed for oral, lingual, sublingual, buccal, and intrabuccal administration can be made without undue experimentation by means well known in the art, for example with an inert diluent or with an edible carrier. The compositions may be enclosed in gelatin

capsules or compressed into tablets. For the purpose of oral therapeutic administration, the pharmaceutical compositions of the present invention may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

Tablets, pills, capsules, troches and the like may also contain binders, recipients, disintegrating agent, lubricants, sweetening agents, and flavoring agents. Some examples of binders include microcrystalline cellulose, gum tragacanth or gelatin. Examples of excipients include starch or lactose. Some examples of disintegrating agents include alginic acid, corn starch and the like. Examples of lubricants include magnesium stearate or potassium stearate. An example of a glidant is colloidal silicon dioxide. Some examples of sweetening agents include sucrose, saccharin and the like. Examples of flavoring agents include peppermint, methyl salicylate, orange flavoring and the like. Materials used in preparing these various compositions should be pharmaceutically pure and nontoxic in the amounts used.

In many of the above-described methods, the GLP-1 (9-36) is formulated in a slow release composition by standard methods, for example a microcrystalline composition.

The GLP-1 (9-36) compositions of the present invention can easily be administered parenterally such as for example, by intravenous, intramuscular, intrathecal or subcutaneous injection, or by subcutaneous infusion pump. Parenteral administration can be accomplished by incorporating the compositions of the present invention into a solution or suspension. Such solutions or suspensions may also include sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Parenteral formulations may also include antibacterial agents such as for example, benzyl alcohol or methyl parabens, antioxidants such as for example, ascorbic acid or sodium bisulfite and chelating agents such as EDTA. Buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose may

also be added. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Rectal administration includes administering the GLP-1 (9-36) pharmaceutical compositions into the rectum or large intestine. This can be accomplished using suppositories or enemas. Suppository formulations can easily be made by methods known in the art. For example, suppository formulations can be prepared by heating glycerin to about 120° C., dissolving the composition in the glycerin, mixing the heated glycerin after which purified water may be added, and pouring the hot mixture into a suppository mold.

Transdermal administration includes percutaneous absorption of the composition through the skin. Transdermal formulations include patches (such as the well-known nicotine patch), ointments, creams, gels, salves and the like.

The present invention includes nasally administering to the mammal a therapeutically effective amount of the composition. As used herein, nasally administering or nasal administration includes administering the composition to the mucous membranes of the nasal passage or nasal cavity of the patient. As used herein, pharmaceutical compositions for nasal administration of a composition include therapeutically effective amounts of the composition prepared by well-known methods to be administered, for example, as a nasal spray, nasal drop, suspension, gel, ointment, cream or powder. Administration of the composition may also take place using a nasal tampon or nasal sponge.

The GLP-1 (9-36) compositions can also be administered to the mammal with at least one other treatment for inhibiting the effects of diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance. One example of such treatments is administration of insulin. Various other treatments are discussed in U.S. Provisional Patent App. No. 60/474,520, incorporated herein by reference.

Another example of a treatment that can be administered with the GLP-1 (9-36) composition is a treatment that inhibits poly(ADP-ribose) polymerase (PARP) activity or accumulation in the mammal. It is known that

hyperglycemia-induced mitochondrial superoxide overproduction activates poly (ADP-ribose) polymerase (PARP). PARP activation, in turn, inhibits glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity which activates at least three of the major pathways of hyperglycemic damage in endothelial cells. Inhibiting PARP activity thus inhibits the development of complications of diabetes. See U.S. Provisional Patent App. No. 60/474,520. Such treatments include administration of a PARP inhibitor. Nonlimiting examples of PARP inhibitors include PJ34, 3-aminobenzamide, 4-amino-1,8-naphthalimide, 6(5H)-phenanthridinone, benzamide, INO-1001, and NU1025. PARP activity can also be inhibited by administering to the mammal a nucleic acid or mimetic that specifically inhibits transcription or translation of the PARP gene. Examples of such nucleic acids or mimetics include an antisense complementary to mRNA of the PARP gene, a ribozyme capable of specifically cleaving the mRNA of the PARP gene, and an RNAi molecule complementary to a portion of the PARP gene. PARP activity can also be inhibited by administration of a compound that specifically binds to the PARP, such as an antibody or an aptamer.

An additional example of a treatment that can be administered with the GLP-1 (9-36) composition is a treatment that activates transketolase in the mammal. See U.S. Provisional Patent App. No. 60/474,520. A preferred method of activating transketolase is by administering a lipid-soluble thiamine derivative to the mammal. Examples of such lipid-soluble thiamine derivatives are benfotiamine, thiamine propyl disulfide, and thiamine tetrahydrofurfuryl disulfide.

Another treatment that can be administered with the GLP-1 (9-36) composition is a treatment that further reduces superoxide in the mammal. Such treatments include administration of an α-lipoic acid, a superoxide dismutase mimetic or a catalase mimetic. Examples of superoxide dismutase mimetics and catalase mimetics include MnTBAP, ZnTBAP, SC-55858, EUK-134, M40403, AEOL 10112, AEOL 10113 and AEOL 10150.

A further treatment that can be administered with the GLP-1 (9-36) composition is a treatment that inhibits excessive release of free fatty acids in the mammal. See U.S. Provisional Patent App. No. 60/474,520. Examples of treatments that inhibit excessive release of free fatty acids are the administration of compounds such as a thiazolidinedione, nicotinic acid, adiponectin and acipimox.

In other embodiments, the invention is directed to methods of inhibiting the development of disease due to diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance in a mammal, or conditions resulting therefrom. The methods comprise treating the mammal with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal. These methods would be expected to be effective in any mammal, including humans.

Nonlimiting examples of diseases that are inhibited by these methods include atherosclerotic, microvascular, or neurologic disease, such as coronary disease, myocardial infarction, atherosclerotic peripheral vascular disease, cerebrovascular disease, stroke, retinopathy, renal disease, neuropathy, and cardiomyopathy.

As with the previously described methods, the GLP-1 (9-36) composition of these methods can also be administered with at least one other treatment for inhibiting the effects of diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance. Such methods have been described above, and in U.S. Provisional Patent App. No. 60/474,520.

In normal animals and people, the endothelial cell enzyme prostacyclin synthase prevents excessive platelet aggregation, and has a variety of other anti-atherogenic actions. Prostacyclin synthase can also protect against development of hypoxic pulmonary hypertension (Geraci et al., 1999). In addition, loss of prostacyclin synthase shifts arachadonic acid metabolism

toward increased thromboxaneA2, lipoxygenase, etc., which have further adverse effects on vessel. The inventor has discovered that treatment with GLP-1 (9-36) protects prostacyclin synthase from hyperglycemia-induced reactive oxygen formation, and is thus a useful treatment for maintaining active prostacyclin synthase. See Example 3.

Thus, in additional embodiments, the invention is directed to methods of reducing hyperglycemia-induced or free fatty acid-induced inactivation of prostacyclin synthase in a mammal. The methods comprise treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal.

In some preferred embodiments, the mammal treated in these methods has or is at risk for hypoxic pulmonary hypertension. In other preferred embodiments, the mammal is at risk for undergoing an acute thrombotic event such as a stroke or a heart attack.

As shown in Example 2, treatment with GLP-1 (9-36) also beneficially reduces hyperglycemia- or free fatty acid- induced decrease in nitric oxide syntherase (eNOS). Normal endothelial production of nitric oxide plays an important role in preventing vascular disease. In addition to its function as an endogenous vasodilator, nitric oxide released from endothelial cells is a potent inhibitor of platelet aggregation and adhesion to the vascular wall. Endothelial NO also controls the expression of genes involved in atherogenesis. It decreases expression of the chemoattractant protein MCP-1, and of surface adhesion molecules such as CD11/CD18, P-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Endothelial cell nitric oxide also reduces vascular permeability, and decreases the rate of oxidation of low density lipoprotein to its pro-atherogenic form. Finally, endothelial cell nitric oxide inhibits proliferation of vascular smooth muscle cells. Endothelium-dependent vasodilation is impaired in both microcirculation and macrocirculation during acute hyperglycemia in normal subjects as well as

in diabetic patients, suggesting that nitric oxide synthase activity may be chronically impaired in diabetic patients.

Thus, the present invention is also directed to methods of inhibiting hyperglycemia-induced or free fatty acid-induced decrease in endothelial nitric oxide synthetase (eNOS) activity in an endothelial cell. The methods comprise treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced decrease in eNOS activity in the cell. As with the analogous methods described above relating to reactive oxygen, the endothelial cell can be part of the vascular tissue of a living mammal, preferably a human. In preferred embodiments, the living mammal has or is at risk for having diabetes, impaired glucose intolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance.

Also as with the methods described above relating to reactive oxygen, any GLP-1 (9-36) form having the sequence of any of SEQ ID NO:3-16 can be utilized with these methods to provide a longer lasting peptide composition.

The invention is also directed to novel forms of GLP-1 (9-36), for example the sequences of SEQ ID NOs:3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16. Preferably, the novel GLP-1 (9-36) is isolated and purified. Where these novel forms of GLP-1 (9-36) are used therapeutically, they are usefully formulated in a pharmaceutically acceptable excipient, and are also preferably an amide.

Examples of these novel forms of GLP-1 (9-36) include a GLP-1 (9-36) that further comprises an additional Arg at the carboxy terminus; a GLP-1 (9-36) that comprises at least one acetylated lysine, for example where the acetyl group is a myristoyl group.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the

specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

Example 1. GLP-1 (9-36) prevents hyperglycemia-induced reactive oxygen production in vascular endothelial cells.

Cultured vascular endothelial cells were treated with GLP-1 (9-36) to determine the effect of GLP-1 (9-36) on hyperglycemia-induced reactive oxygen production by those cells.

Materials and Methods

Cell culture conditions. For ROS measurement, bovine aortic endothelial cells (BAECs, passage 4-10) were plated in 96 well plates at 100,000 cells /well in Eagle's MEM containing 10% FBS, essential and nonessential amino acids, and antibiotics. Cells were incubated with either 5 mM glucose, 30 mM glucose, 30 mM glucose plus 10 nM GLP-1 (7-36), 30 mM glucose plus 10 nM GLP-1 (7-36), 30 mM glucose plus 10 nM GLP-1 (7-36) plus 10 μM pyrrolidide (a DPP IV inhibitor), 30 mM glucose plus 10 nM GLP-1 (7-36) plus 10 μM pyrrolidide and 100 μM phosphoaramidon (a neutral endopeptidase 24.11 inhibitor), and 30 mM glucose plus GLP-1 (9-36) plus 10 nM exendin 9-39, a blocker of the GLP-1 (7-36) receptor. The pyrrolidide, phosphoramidon, and exendin 9-39 were each added to the cells four hours before the addition of the peptides. The ROS measurements were performed 24 hrs later.

Intracellular reactive oxygen species measurements. The intracellular formation of reactive oxygen species was detected using the fluorescent probe CM-H_2DCFDA (Molecular Probes). Cells ($1x10^5$ ml^{-1}) were loaded with 10 μM CM-H_2DCFDDA, incubated for 45 min at 37 °C, and analysed in an HTS 7000 Bio Assay Fluorescent Plate Reader (Perkin Elmer) using the HTSoft program. ROS production was determined from an H_2O_2 standard curve (10-200 nmol ml^{-1}).

Results and Discussion

As shown in FIG. 1, GLP-1 (9-36) inhibited production of ROS in vascular endothelial cells in culture. Diabetic levels of hyperglycemia cause increased ROS (superoxide) production in these cells (FIG. 1, bar 2). Adding GLP-1 (7-36) completely prevents this damaging effect (FIG. 1, bar 3). However, when GLP-1 degradation is blocked by enzyme inhibitors (FIG. 1, bars 4 and 5), the intact GLP-1 (7-36) has no effect on hyperglycemia-induced ROS.

In contrast, addition of the "inactive" GLP-1 degradation product (FIG. 1, bar 6), completely inhibits hyperglycemia-induced overproduction of ROS. Furthermore, blockade of the GLP-1 receptor with e9-39 has no effect on this property, strongly suggesting that the effect is mediated through a different, undiscovered receptor.

Thus, the degradation product of GLP-1, previously thought to be biologically inactive, has a profound effect on vascular endothelial cells---it prevents completely hyperglycemia-induced overproduction of superoxide (FIG. 1).

Example 2. GLP-1 (9-36) prevents hyperglycemia-induced decreases in endothelial nitric oxide synthase (eNOS) activity in vascular endothelial cells.

Cultured vascular endothelial cells were treated with GLP-1 (9-36) to determine the effect of GLP-1 (9-36) on hyperglycemia-induced decreases in eNOS activity in those cells.

Materials and Methods

Cell-culture conditions. For measurement of endothelial nitric oxide activity (eNOS), bovine aortic endothelial cells (BAECs, passage 4-10) were plated in 24 well plates at 200,000 cells /well in Eagle's MEM containing 10% FBS, essential and nonessential amino acids, and antibiotics. Cells were incubated with either 5 mM glucose, 30 mM glucose , 30 mM glucose plus 10 nM GLP-1 (7-36), 30 mM glucose plus 10 nM GLP-1 (7-36) plus 10 μM pyrrolidide (a DPP IV inhibitor) (not shown in FIG. 2), 30 mM glucose plus 10

nM GLP-1 (7-36) plus 10 μM pyrrolidide and 100 μM phosphoaramidon (a neutral endopeptidase 24.11 inhibitor), 30 mM glucose + GLP-1 (9-36), and 30 mM glucose plus GLP-1 (9-36) plus 10 nM exendin 9-39, a blocker of the GLP-1 (7-36) receptor. The pyrrolidide, phosphoramidon, and exendin 9-39 were each added to the cells four hours before the addition of the peptides. eNOS activity measurements were performed 48 hrs later.

Measurement of eNOS activity. eNOS activity in cells was determined by first incubating cells in L-arginine-deficient, serum-free MEM media for 6 hours. This media was then replaced with PBS buffer containing 120 mM NaCl, 4.2 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.2 mM Na_2HPO_4, 0.37 mM KH_2PO_4, 10 mM HEPES, and 7.5 mM glucose (500 μl/well); cells were then incubated for 15 minutes at 37 °C. The eNOS activity assay was initiated by incubating cells with PBS buffer (400 μl/well) containing 1.5 Ci/ml [^{3}H]L-arginine for 15 minutes. The reaction was stopped by adding 1 N ice-cold TCA (500 μl/well). Cytosol preparations were transferred to ice-cold silanized glass tubes and extracted three times with water-saturated ether. The samples were neutralized with 1.5 ml of 25 mM HEPES (pH 8.0) and applied to Dowex AG50WX8 columns (Tris form) (Sigma Chemical Co., St. Louis, Missouri, USA). Columns were eluted with 1 ml of 40 mM HEPES buffer (pH 5.5) containing 2 mM EDTA and 2 mM EGTA. The eluate was collected in glass scintillation vials for [3H]L-citrulline quantitation by liquid scintillation spectroscopy.

Results and Discussion

The results are summarized in FIG. 2. Diabetic levels of hyperglycemia cause decreased eNOS production in these cells (FIG. 2, bar 2). Adding GLP-1 (7-36) completely prevents this damaging effect (FIG. 2, bar 3). However, when GLP-1 (7-36) degradation is blocked by enzyme inhibitors (FIG. 2, bar 4), the intact GLP-1 (7-36) has no effect on hyperglycemia-induced eNOS.

In contrast, addition of GLP-1 (9-36) (FIG. 2, bar 5), completely inhibits hyperglycemia-induced overproduction of ROS. Furthermore, blockade of the GLP-1 receptor with e9-39 (FIG. 2, bar 6) has no effect on this property,

providing further evidence that the effect is mediated through a different, undiscovered receptor.

These results precisely mirrored the results with ROS discussed in Example 1, indicating a common mechanism.

Example 3. GLP-1 (9-36) prevents diabetes-induced inactivation/inhibition of prostacyclin synthase in diabetic mouse aortas.

In vivo studies were conducted to determine whether GLP-1 (9-36) has a physiologically relevant *in vivo* effect on prostacyclin synthase, which is strongly affected by reactive oxygen.

Materials and Methods

Animal studies. Male C57Bl6 mice (6-8 weeks old) were made diabetic by daily injections of 50 mg/kg streptozotocin in 0 .05 M NaCitrate pH 4.5 after an eight hour fast, for five consecutive days. Two weeks after the initial injection the blood glucose was determined and the diabetic mice were randomized into two groups with equal mean blood glucose levels. Alzet micro-osmotic pumps were inserted into 10 diabetic mice. The pump was filled with GLP-1 (9-36) peptide at a concentration of 10 μg/100 μl. Seven days later 10 untreated diabetic mice, 10 treated diabetic mice, and 10 non-diabetic control mice were sacrificed. Blood glucose was determined at time of sacrifice. The aorta was removed from the abdominal bifurcation to the aortic arch, and prostacyclin activity was determined by measurement of its stable product 6-keto-$PGF_{1\alpha}$.

Measurement of 6-keto-$PGF_{1\alpha}$. 6-keto-$PGF_{1\alpha}$ is a stable product which is produced by the non-enzymatic hydration of PGI_2. A competitive immunoassay method (Correlate-EIA) was used for the quantitative determination of 6-keto-$PGF_{1\alpha}$. The samples were preparing from dissected mouse aortas. The aorta was dissected from the abdominal bifurcation to the aortic arch. Briefly, the aorta was washed with PBS and incubated at 37 °C for 3 hours in 400 μl incubation buffer which contained 20 mM Tris-HCl buffer (pH 7.5) and 15 μl

arachidonic acid. 100 µl of sample was used to measure the 6-keto-PGF1a concentration according to the manufacturer's instructions (Assay Design Inc.). The data are expressed per aorta.

Results and Discussion

The results are summarized in FIG. 3. GLP-1 (9-36) ("Peptide") completely eliminated the diabetes-induced inactivation of prostacyclin synthase. This shows that *in vivo* administration of GLP-1 (9-36) has a significant effect on diabetes-induced reactive oxygen formation and physiological systems affected by reactive oxygen.

Example 4. GLP-1 (9-36) prevents hyperglycemia-induced reactive oxygen production in hepatocytes.

An experiment similar to that described in Example 1 was performed, using hepatocytes rather than endothelial cells. As shown in FIG. 4, GLP-1 (9-36) inhibited hyperglycemia-induced reactive oxygen (ROS) formation in hepatocytes in a similar manner as with endothelial cells.

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

Appendix - SEQ ID Nos

SEQ ID NO:1 GLP-1(9-36) Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg

SEQ ID NO:2 GLP-1 (9-37) Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly

SEQ ID NO:3 GLP-1 (9-36 + arg37) Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Arg

SEQ ID NO:4 GLP-1 (9-36) acyl-Lys26 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg

SEQ ID NO:5 GLP-1 (9-37) acyl-Lys26 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly

SEQ ID NO:6 GLP-1 (9-36) acyl-Lys26 + arg 37 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Arg

SEQ ID NO:7 GLP-1 (9-36) acyl-Lys34 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg

SEQ ID NO:8 GLP-1 (9-37) acyl-Lys34 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Gly

SEQ ID NO:9 GLP-1 (9-36) acyl-Lys34 + arg 37 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Arg

SEQ ID NO:10 GLP-1 (9-36) acyl-Lys34 and acyl-Lys26 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg

SEQ ID NO:11 GLP-1 (9-36) acyl-Lys34 and acyl-Lys26 + arg 37 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Arg

SEQ ID NO:12 GLP-1 (9-37) acyl-Lys34 and acyl-Lys26 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Gly

SEQ ID NO:13 GLP-1 (9-37) + Arg38 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Arg

SEQ ID NO:14 GLP-1 (9-37) acyl-Lys34 + Arg38 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Gly Arg

SEQ ID NO:15 GLP-1 (9-37) acyl-Lys26 + Arg38 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Arg

SEQ ID NO:16 GLP-1 (9-37) acyl-Lys34 and acyl-Lys26 + Arg38 Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala acLys Glu Phe Ile Ala Trp Leu Val acLys Gly Arg Gly Arg

What is claimed is:

1. A method of inhibiting hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in a mammalian cell, the method comprising treating the cell with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the cell.

2. The method of claim 1, wherein the reactive oxygen formation is hyperglycemia-induced.

3. The method of claim 1, wherein the cell is in a living mammal.

4. The method of claim 1, wherein the cell is selected from the group consisting of a nerve cell, a renal mesangial cell, a β cell, an adipocyte, an endothelial cell or a hepatocyte.

5. The method of claim 1, wherein the cell is an endothelial cell.

6. The method of claim 5, wherein endothelial cell is a vascular endothelial cell.

7. The method of claim 5, wherein the endothelial cell is in a mammal that has or is at risk for having diabetes, impaired glucose intolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance.

8. The method of claim 5, wherein the mammal is critically ill.

9. The method of claim 5, wherein the mammal has chronic ischemia.

10. The method of claim 1, wherein the cell is a hepatocyte.

11. The method of claim 10, wherein the hepatocyte is in a living mammal that has or is at risk for ischemia/reperfusion injury, endotoxin injury, or alcoholic liver disease.

12. The method of claim 1, wherein the cell is a β cell.

13. The method of claim 12, wherein the β cell is in a living mammal that has or is at risk for impaired glucose-stimulated insulin secretion.

14. The method of claim 1, wherein the cell is a neuron.

15. The method of claim 14, wherein the neuron is a peripheral neuron.

16. The method of claim 1, wherein the cell is an adipocyte.

17. The method of claim 1, wherein the cell is a renal mesangial cell.

18. The method of claim 1, wherein the GLP-1 (9-36) has the sequence of SEQ ID NO:1.

19. The method of claim 1, wherein the GLP-1 (9-36) is an amide.

20. The method of claim 1, wherein the GLP-1 (9-36) further comprises an additional amino acid at the carboxy terminus.

21. The method of claim 20, wherein the additional amino acid is a Gly.

22. The method of claim 20, wherein the additional amino acid is an arginine.

23. The method of claim 1, wherein the GLP-1 (9-36) has the sequence of any one of SEQ ID NOs:2-16.

24. The method of claim 23, where the GLP-1 (9-36) further has an additional Arg at the carboxy terminus.

25. The method of claim 1, further comprising monitoring hyperglycemia-induced reactive oxygen formation after treatment with the GLP-1 (9-36) composition.

26. The method of claim 25, wherein the reactive oxygen formation is monitored by directly measuring reactive oxygen in the cell.

27. The method of claim 25, wherein the reactive oxygen formation is monitored by measuring prostacyclin synthase activity in the cell.

28. The method of claim 27, wherein the prostacyclin synthase activity is measured by measuring formation of 6-keto-$PGF_{1\alpha}$.

29. The method of claim 3, wherein the GLP-1 (9-36) composition is administered parenterally.

30. The method of claim 3, wherein the GLP-1 (9-36) composition is administered intravenously.

31. The method of claim 3, wherein the GLP-1 (9-36) composition is administered by a subcutaneous infusion pump.

32. The method of claim 3, wherein the mammal is administered at least one other treatment for inhibiting the effects of diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance.

33. The method of claim 32, wherein the at least one other treatment is administration of insulin.

34. The method of claim 32, wherein the at least one other treatment inhibits poly(ADP-ribose) polymerase (PARP) activity or accumulation in the mammal.

35. The method of claim 34, wherein the PARP activity is inhibited by administering to the mammal a PARP inhibitor.

36. The method of claim 35, wherein the PARP inhibitor is selected from the group consisting of PJ34, 3-aminobenzamide, 4-amino-1,8-naphthalimide, 6(5H)-phenanthridinone, benzamide, INO-1001, and NU1025.

37. The method of claim 35, wherein the PARP inhibitor is selected from the group consisting of PJ34, INO-1001, and 3-aminobenzamide.

38. The method of claim 34, wherein the PARP activity is inhibited by administering to the mammal a nucleic acid or mimetic that specifically inhibits transcription or translation of the PARP gene.

39. The method of claim 38, wherein the nucleic acid or mimetic is selected from the group consisting of an antisense complementary to mRNA of the PARP gene, a ribozyme capable of specifically cleaving the mRNA of the PARP gene, and an RNAi molecule complementary to a portion of the PARP gene.

40. The method of claim 34, wherein the PARP activity is inhibited by administration of a compound that specifically binds to the PARP.

41. The method of claim 40, wherein the compound that specifically binds to the PARP is an antibody or an aptamer.

42. The method of claim 32, wherein the at least one other treatment activates transketolase in the mammal.

43. The method of claim 42, wherein transketolase is activated by administering a lipid-soluble thiamine derivative to the mammal.

44. The method of claim 43, wherein the lipid-soluble thiamine derivative is selected from the group consisting of benfotiamine, thiamine propyl disulfide, and thiamine tetrahydrofurfuryl disulfide.

45. The method of claim 32, wherein the at least one other treatment further reduces superoxide in the mammal.

46. The method of claim 45, wherein the superoxide is reduced in the mammal by administering to the mammal a compound selected from the group consisting of an α-lipoic acid, a superoxide dismutase mimetic and a catalase mimetic.

47. The method of claim 45, wherein the compound is a superoxide dismutase mimetic or a catalase mimetic selected from the group consisting of MnTBAP, ZnTBAP, SC-55858, EUK-134, M40403, AEOL 10112, AEOL 10113 and AEOL 10150.

48. The method of claim 47, wherein the compound is selected from the group consisting of M40403, MnTBAP, AEOL 10112, AEOL 10113, AEOL 10150, and ZnTBAP.

49. The method of claim 32, wherein the at least one other treatment inhibits excessive release of free fatty acids in the mammal.

50. The method of claim 49, wherein excessive release of free fatty acids is inhibited by administering to the mammal a compound selected from the group consisting of a thiazolidinedione, nicotinic acid, adiponectin and acipimox.

51. A method of inhibiting the development of disease due to diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance in a mammal, or conditions resulting therefrom, the method comprising treating the mammal with a pharmaceutically acceptable composition comprising GLP-1 (9-36) sufficient to inhibit hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal.

52. The method of claim 51, wherein the disease is an atherosclerotic, microvascular, or neurologic disease.

53. The method of claim 51, wherein the disease is selected from the group consisting of coronary disease, myocardial infarction, atherosclerotic peripheral vascular disease, cerebrovascular disease, stroke, retinopathy, renal disease, neuropathy, and cardiomyopathy.

54. The method of claim 51, wherein the mammal is administered at least one other treatment for inhibiting the effects of diabetes, impaired glucose tolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance.

55. A method of reducing hyperglycemia-induced or free fatty acid-induced inactivation of prostacyclin synthase in a mammal, the method comprising treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in the mammal.

56. The method of claim 55, wherein the mammal has or is at risk for hypoxic pulmonary hypertension.

57. The method of claim 55, wherein the mammal is at risk for undergoing an acute thrombotic event.

58. The method of claim 57, wherein the acute thrombotic event is a stroke or a heart attack.

59. A method of inhibiting hyperglycemia-induced or free fatty acid-induced decrease in endothelial nitric oxide synthetase (eNOS) activity in an endothelial cell, the method comprising treating the mammal with GLP-1 (9-36) sufficient to inhibit the hyperglycemia-induced or free fatty acid-induced decrease in eNOS activity in the cell.

60. The method of claim 59, wherein the endothelial cell is part of the vascular tissue of a living mammal.

61. The method of claim 60, wherein the living mammal has or is at risk for having diabetes, impaired glucose intolerance, stress hyperglycemia, metabolic syndrome, and/or insulin resistance.

62. The method of any one of claims 1-61, wherein the GLP-1 (9-36) is formulated in a slow release composition.

63. The method of claim 62, wherein the slow release composition is a microcrystalline composition.

64. The method of claim 62, wherein the GLP-1 (9-36) sequence is altered to form a more slow-release composition than the GLP-1 of SEQ ID NO:1.

65. The method of claim 64, wherein GLP-1 (9-36) sequence is selected from the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16.

66. The method of claim 64, wherein the GLP-1 (9-36) sequence comprises at least one acetylated lysine where the acetyl group is a myristoyl group.

67. An isolated and purified GLP-1 (9-36) consisting essentially of a sequence selected from the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16.

68. The GLP-1 (9-36) of claim 67, wherein the GLP-1 (9-36) is an amide.

69. The GLP-1 (9-36) of claim 67, wherein the GLP-1 (9-36) further comprises an additional Arg at the carboxy terminus.

70. The GLP-1 (9-36) of claim 67, wherein the GLP-1 (9-36) sequence comprises at least one acetylated lysine where the acetyl group is a myristoyl group.

71. A composition comprising the GLP-1 (9-36) of claim 67 in a pharmaceutically acceptable excipient.

Abstract

Methods of inhibiting hyperglycemia-induced or free fatty acid-induced reactive oxygen formation in mammalian cells and mammals using the degradation product of glucagon-like peptide 1, GLP-1 (9-36) are provided. Various GLP-1 (9-36) compositions are also provided.

FIG. 1

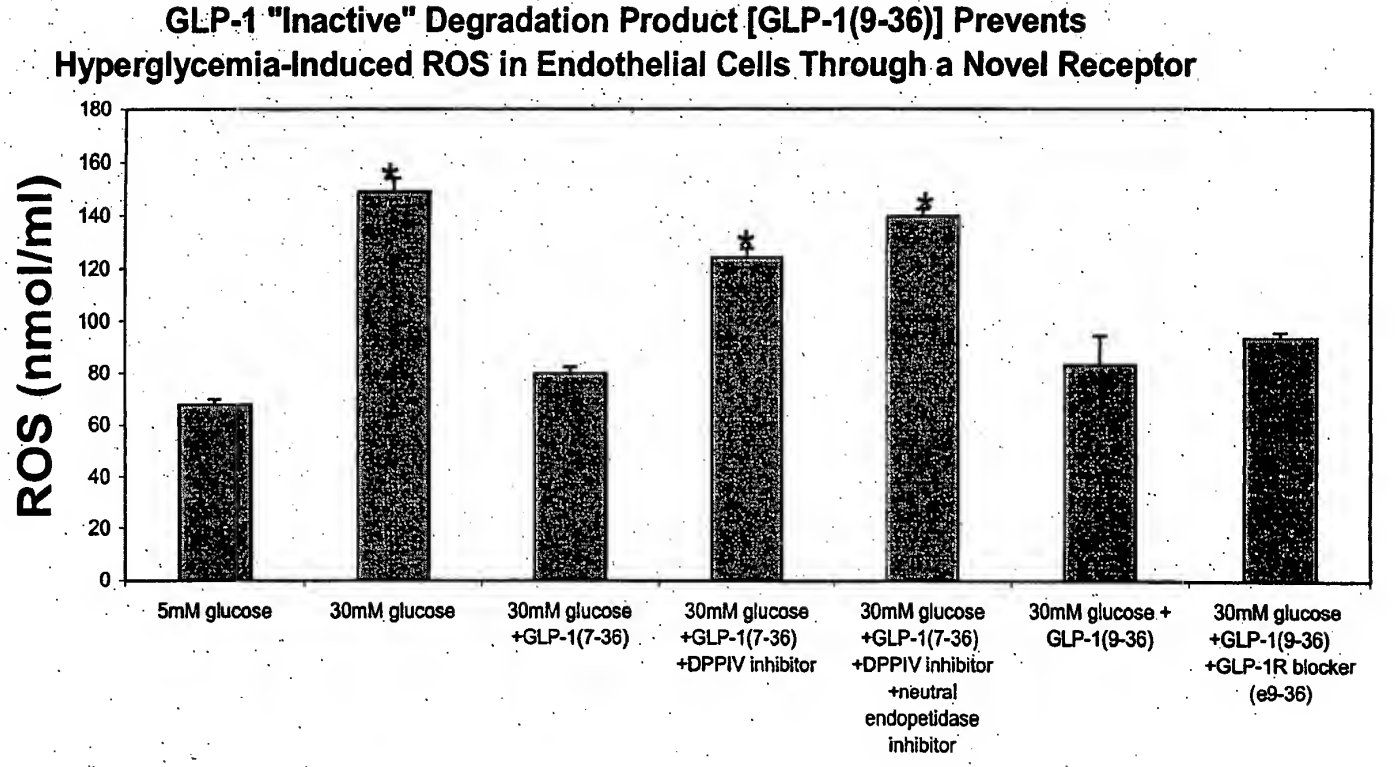

FIG. 2

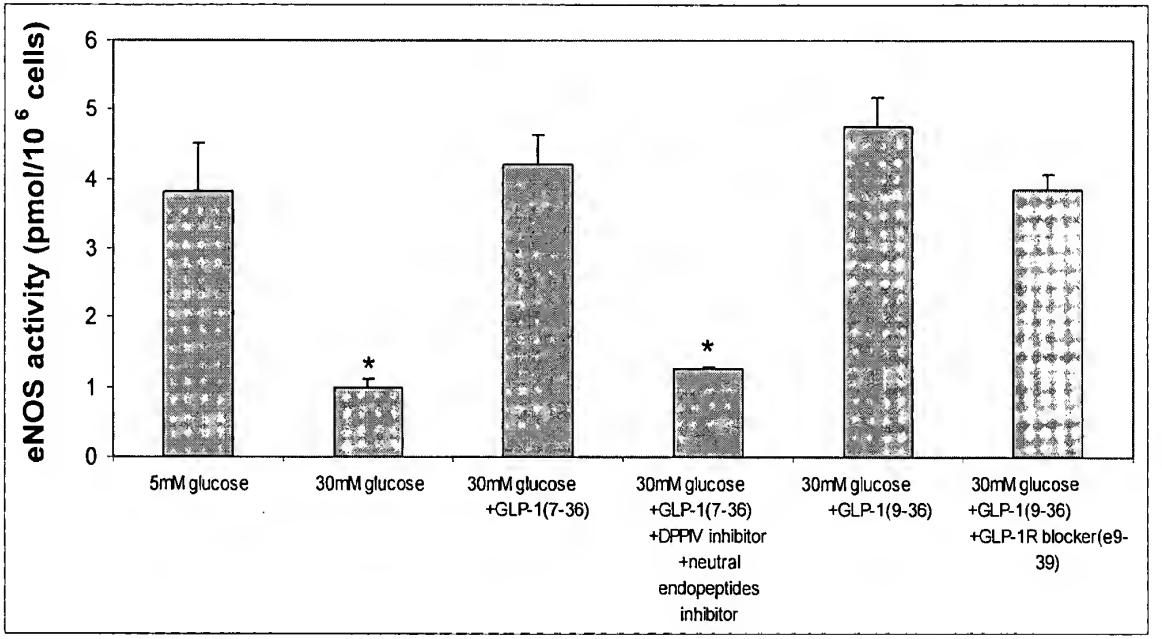

FIG. 3

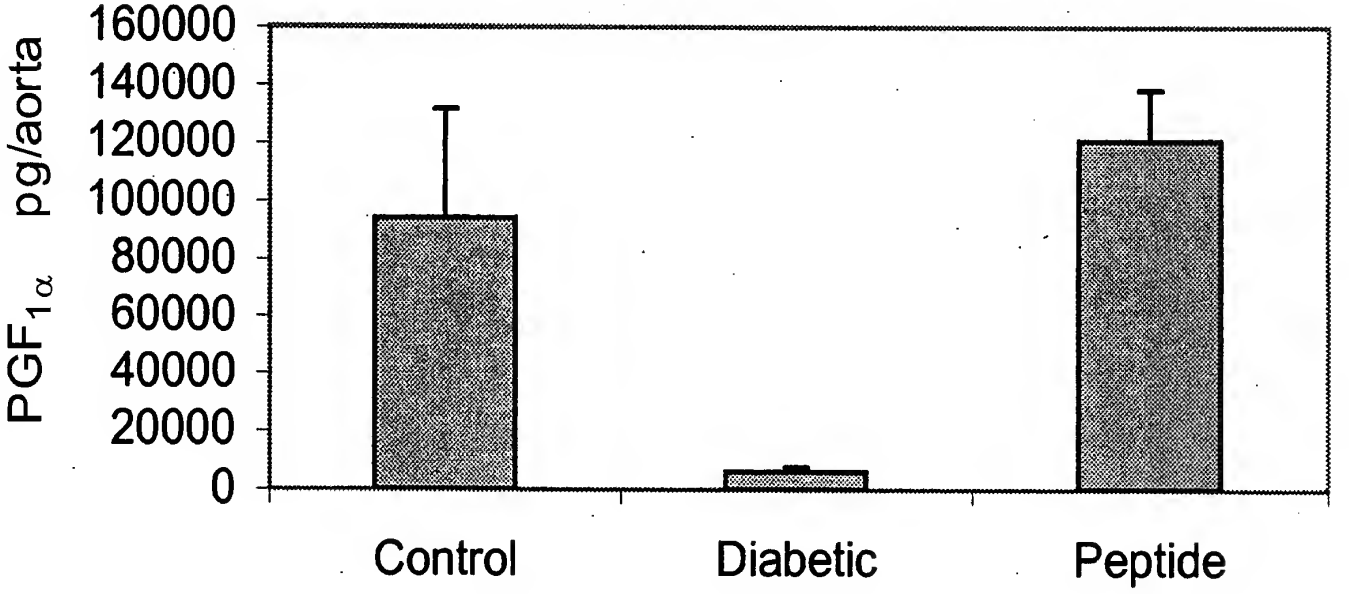

FIG. 4